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REVIEW

Barx1 and evolutionary changes in feeding

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Abstract

During mouse embryonic development, the *Barx1* homeobox gene is expressed in the mesenchymal cells of molar teeth and stomach. During early stages of molar development, *Barx1* has an instructive role, directing the as yet undetermined ectomesenchymal cells in the proximal region of the jaws to follow a multicuspid tooth developmental pathway. We review here recent results showing an absence of stomach tissue in *Barx1* mutant mice. The data strongly suggest that in the presumptive stomach mesenchyme *Barx1* acts to attenuate Wnt signalling allowing digestive tract endoderm to differentiate into a highly specialized stomach epithelium. In the light of these new data, we discuss the possibility that evolutionary changes in the *Barx1* gene could have simultaneously altered the dentition and the digestive system, therefore positioning *Barx1* as a key gene in the evolution of mammals.

Key words *Barx1*; homeobox; stomach; teeth.

The origins of the first oral teeth that appear during the evolution of vertebrates have been the subject of much controversy. Histological similarities between the denticles of the dermal armour and the pharyngeal teeth of both extinct and extant fishes have led to two opposing views: (1) that teeth evolved from internalization of dermal denticles into the mouth, and (2) that pharyngeal teeth 'moved' rostrally to the oral cavity (Smith & Coates, 1998). Regardless of the actual mechanism, the first teeth to form had a simple conical shape similar to those found in many aquatic species today such as crocodilians and dolphins. This simple tooth shape provides an efficient mechanism for obtaining food but not to process it before swallowing, because only biting and tearing are possible. The time taken for the metabolic benefits of digestion (including energy) to be released from the food is dependent on the amount ingested and on the time taken for the stomach to break down the complex elements of the diet.

The gradual evolution of increasingly complex tooth crown shapes in the mammal-like reptiles, in particular the formation of cusps and occlusion of the apposing teeth in the cynodonts, enabled food to be assimilated more rapidly, allowing maintenance of a high metabolic rate (Kemp, 1982, and references therein). The dentition became differentiated into food-gathering incisors anteriorly and complex food-processing postcanine teeth posteriorly, so that food could be sheared and crushed in addition to the more ancient piercing function. Associated changes in the jaw and tongue musculature facilitated use of the molar teeth for breaking down food in the oral cavity, and hence more rapid digestion in the stomach and intestine. This advance enabled animals to develop endothermy and adopt a greater variety of feeding strategies, leading to new and more complex diets and the exploitation of new food niches.

The ability to form multicuspid, occluding teeth that enable food to be ground and chewed more effectively thus represents a pivotal point in premammalian evolution. A key question therefore is whether these changes were associated with changes in the digestive system. New food would presumably have put pressure on any existing digestive system. Could any animal have been successful in exploiting a new feeding niche without significant accompanying changes to its digestive system to allow effective post-oral processing of a new food? In order to attempt to answer this fundamental evolutionary question we have been investigating the

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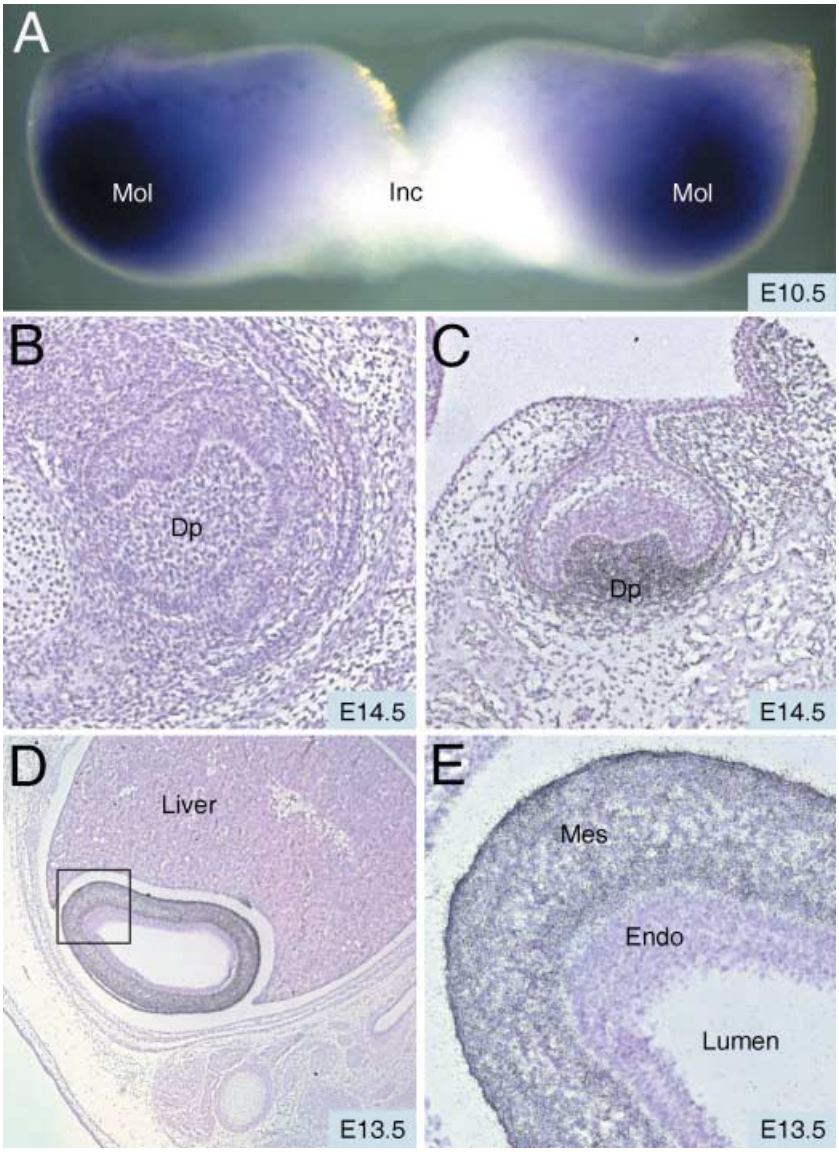

Fig. 1 In mouse embryos, *Barx1* is expressed in the mesenchyme of molar teeth and stomach. (A) At 10.5 days of development, before any morphological sign of tooth development, *Barx1* is expressed in broad proximal mesenchymal domains where the presumptive molars will form. (B) At the cap stage of tooth development, *Barx1* expression is not detected in the developing incisors, but (C) is very strong in the dental papilla (Dp) of the molars. (D) *Barx1* is expressed in the mesenchyme lining the stomach. (E) Enlargement of the area outlined in D. Dp, dental papilla; Endo, endoderm; Inc, incisors; Mes, mesenchyme; Mol, molars. (D,E are reproduced by permission of Cell Press Inc.)

function of genes that are expressed during the early development of both teeth and stomach.

We have identified the *Barx1* homeobox gene as being expressed in the mesenchymal cells during both molar tooth and stomach development in mice. During the development of molars, *Barx1* is first expressed in the predental neural crest derived mesenchyme (ectomesenchyme) in the jaw primordia, in a very restricted group of cells from which molar tooth germs will form (Fig. 1A). During subsequent development, *Barx1* continues to be expressed in molar dental mesenchyme and its derivatives but is never expressed during incisor development (Fig. 1B,C). We investigated the function of *Barx1* during molar tooth development by ectopically expressing *Barx1* in presumptive incisor tooth mesenchyme. This was achieved either by interfering with the regulation of *Barx1* gene expression (Tucker et al. 1998) or more directly by misexpressing *Barx1* via electroporation of a *Barx1* expression constructs (Fig. 2A,B). In each case the same result was obtained, namely that molar-like teeth developed in place of incisors (Fig. 2C,D). *Barx1* is hence a gene that has a direct role in directing predental ectomesenchymal cells to follow a morphogenetic pathway leading to multicuspid tooth development.

Barx1 is also strongly expressed in the mesenchyme of the developing stomach (Fig. 1D,E). In order to address the role of *Barx1* in stomach and tooth development we generated *Barx1* mutant mice using gene targeting. On a 129SvEv background *Barx1*$^{-/-}$ embryos

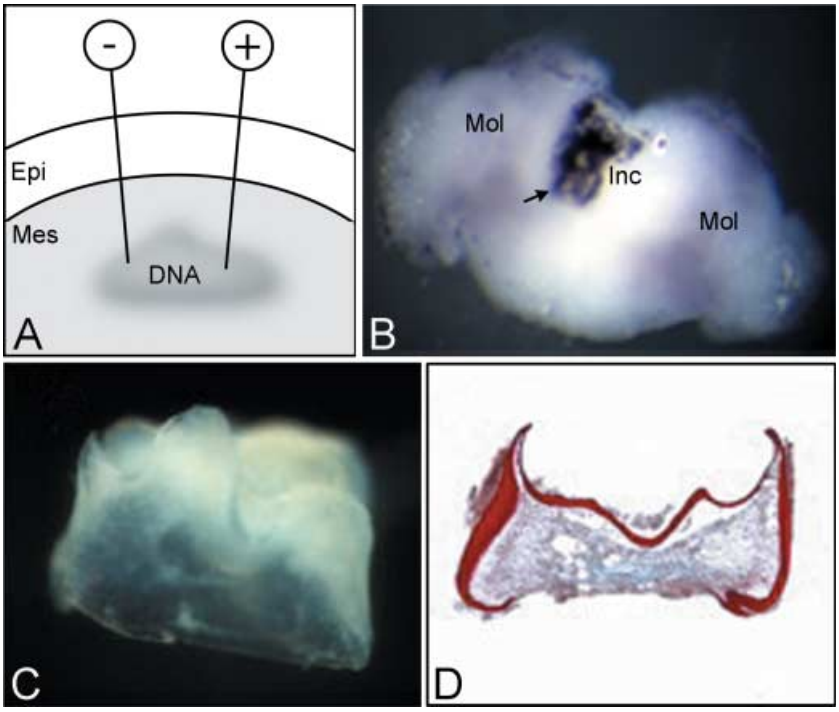

Fig. 2 Misexpression of *Barx1* in the presumptive incisor mesenchyme at E10 results in transformation of tooth identity from incisor to molar. (A) Schematic diagram of electroporation in the mesenchyme. DNA is carefully injected into the incisor mesenchyme. Electrodes are then slightly inserted into the mesenchyme and electric pulses applied. (B) Whole mount *in situ* hybridization for *Barx1* after electroporation of a *Barx1* expression construct into the incisor mesenchyme at E10. (C) Example of a tooth obtained from E10 incisor regions electroporated with a *Barx1* expression construct that were cultured for 1 day *in vitro* and implanted under host kidney capsules. Instead of the typical conical shape of incisors, multicuspid teeth develop. (D) A section of the multicuspid tooth shown in C stained with alcian blue and chlorantine fast red. Epi, epithelium; Inc, incisors; Mes, mesenchyme; Mol, molars.

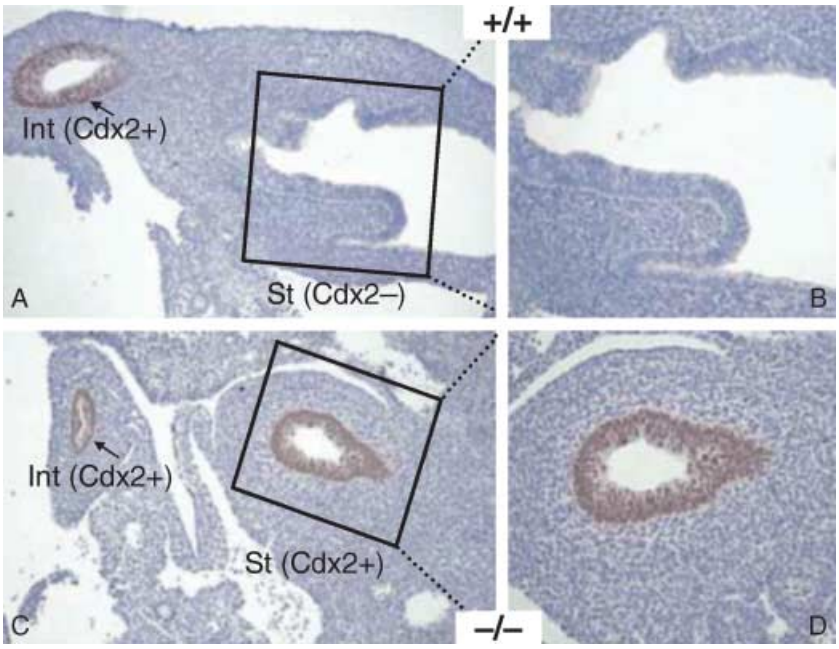

Fig. 3 Immunostaining for the intestinal marker Cdx2 in E12.5 gastrointestinal (GI) tracts of *Barx1* null mouse embryos and control littermates. (A,B) Wild-type embryos. (C,D) *Barx1*$^{-/-}$ littermates. (B,D) Enlargement of areas outlined, respectively, in A and D. In a normal E12.5 GI tract, Cdx2 is present in the epithelium of the intestine (arrow) and absent from the stomach (outlined area). (C) Cdx2 is present in the defective epithelium of *Barx1*$^{-/-}$ stomach (outlined area), in addition to the epithelium of the intestine (arrow). Int, intestine; St, stomach. (Reproduced by permission of Cell Press Inc.)

die at around embryonic day (E)13.5, but analysis of the developing digestive system of these embryos revealed an absence of stomach tissue. The histology of the digestive tract in the region of the stomach suggested that intestine had developed in place of the stomach (Kim et al. 2005). This intestinal identity was confirmed by immunohistochemical staining for Cdx2, a marker of embryonic intestinal epithelium (Fig. 3A–D). In *Barx1*$^{-/-}$ mice, stomach epithelium is transformed into intestine, suggesting that *Barx1* in the stomach mesenchyme is acting to regulate the expression of signalling molecules that direct gastric epithelial differentiation. The mechanism of *Barx1* action in the stomach most likely involves the attenuation of Wnt signalling, as expression of the secreted Wnt antagonists *Sfrp1* and *Sfrp2* is down-regulated in *Barx1* mutant stomach mesenchyme.

Wnt signalling has been identified as an important component of the pathways directing stomach epithelial differentiation (Theodosiou & Tabin, 2003). The morphology of the molar teeth could not be observed because these mutant embryos did not survive beyond E13.

Barx1 is thus expressed in the mesenchymal cells during the development of two different organ primordia. It acts indirectly to control epithelial differentiation and morphogenesis to produce both the specialized epithelium of the stomach and the specific folding patterns of dental epithelium that produce molar cusps. Conceivably, therefore, evolutionary changes in the *Barx1* gene or its regulation could have produced changes in the phenotype of both the teeth and the digestive system. Such co-ordinated changes would have given an animal a distinct selective advantage and thus *Barx1* may have been a key gene in the evolution of mammalian characteristics and feeding behaviour in advanced mammal-like reptiles.

Can *Barx1* expression be equated with tooth and stomach phenotype in non-mammalian species with simple homodont dentitions entirely composed of conical teeth? Did random mutation in the *Barx1* gene, or more likely changes in its mode of regulation, lead to it being able to control dental epithelial folding and stomach epithelial differentiation, an event that was important in the evolution of mammals? These are answerable questions that form the focus of our current research.

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